J. Helminthol. Soc. Wash.
60(1), 1993, pp. 48–54

Ultrastructure of the First-Stage Larvae of a *Philometra* sp. (Nematoda: Philometridae) from Freshwater Drum (*Aplodinotus grunniens*)

ROSMARIE KELLY AND JOHN L. CRITES
Department of Zoology, The Ohio State University, Columbus, Ohio 43210

ABSTRACT. An ultrastructural study of the first-stage larvae of *Philometra* sp., utilizing scanning and transmission electron microscopy, was undertaken to understand behaviors of this larval stage better. Larvae have an egg tooth present on the dorsal labial ridge and may penetrate the gut wall of the copepod host through its use. Internally, first-stage larvae were found to have a partially developed digestive system that is filled with a yolklike material and ends in storage cells. Larvae may not feed at this stage, relying instead on this material for an energy source. The caudal end of the first-stage larva consists of a concave, oval-spatulate structure abundantly supplied with muscle. Muscular contractions possibly allow the attachment to particles seen during the free-swimming phase of this larval stage.

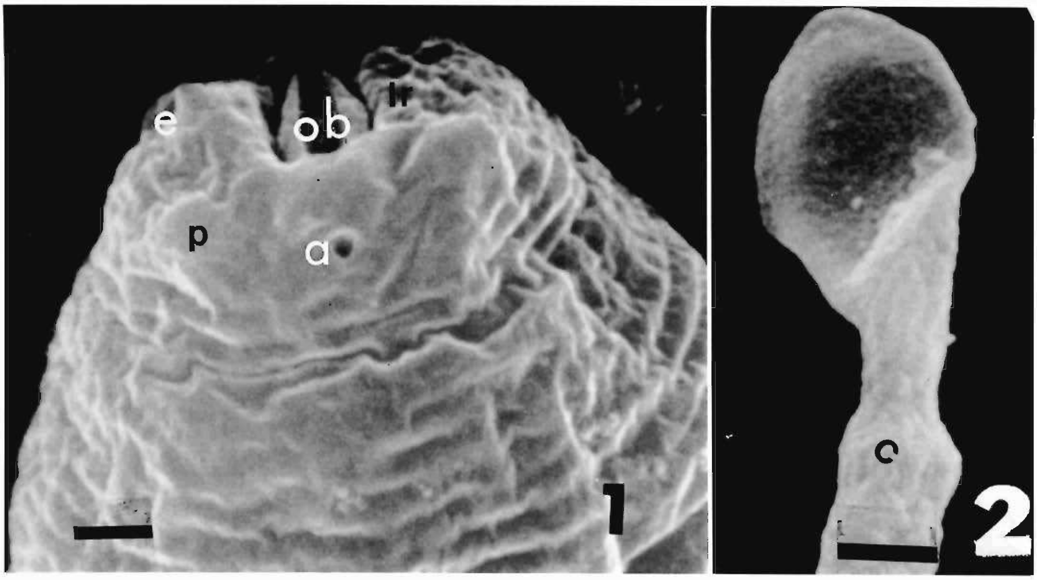
KEY WORDS: *Philometra* sp., Nematoda, morphology, ultrastructure, SEM, TEM.

Nematodes of the family Philometridae produce free-swimming first-stage larvae. From late June to early August, larvigerous females of *Philometra* sp. can be found streaming from the eyes of the freshwater drum. These worms rupture, releasing thousands of first-stage larvae into the water. The larvae attach in groups to particles and are ingested by the intermediate host, cyclopoid copepods. Once within the copepod, the larvae penetrate the copepod gut wall and establish themselves in the hemocoel. They undergo 2 molts in the hemocoel, developing to infective third-stage larvae. If the copepod is then ingested by a young freshwater drum, the larvae undergo 2 more molts, migrate to the eye of the fish, and establish themselves. Copulation occurs in the ocular orbits, and the males subsequently die. Fertilized ova within the females developed into first-stage larvae the following summer (Crites, 1980).

During its life cycle, *Philometra* sp. undergoes extensive internal and external change. Although some work has been conducted on adult philometrids using light microscopy, almost nothing is known about the structure of the larval stages. There are a few descriptions of first-stage larvae based on evidence from light microscopy, but these descriptions are incomplete and often contradictory. Crites (1980) described the first-stage larvae as having no discernable lips, an open esophageal lumen, an intestine with no discernible lumen, and a long tapering tail with an open cavity at the posteriormost end. *Philometroides,* a related genus, is described as having first-stage larvae with a dilated esophagus ending in 3 distinct cells and an intestine filled with refractile granules (Uhazy, 1976). Uhazy (1976) also mentioned that *Philometroides nodulosa* and *P. sanguinea* do not have terminal buttonlike swellings on the tail, but *P. huronensis* does. Thomas' (1929) description of *P. nodulosa* first-stage larvae included a mouth surrounded by a dorsal and a ventral lip, a thin esophagus with a visible lumen joined to an expanded intestine containing refractile granules, cement glands in the caudal region that opened to the exterior 40 μm from the distal end of the tail, and an anus situated on the ventral surface 48 μm from the posterior end. Krecker (1916) described the larvae of *Filaria cingula* (probably a species of *Philometroides*) as tapering to a sharp hairlike point at the posterior end. First-stage larvae of *Philometra ovata* are described as having a conical tail with a sharply pointed tip (Moravec, 1980).

Although *Philometra* sp. larvae are known to be extremely active, the energy source for this activity is unknown. It is uncertain whether or not this larval stage feeds. Although an anus is present, it is unknown if this structure is functional; the anus disappears in the adult.

It would seem that larval behaviors, such as clumping, penetration of the copepod gut wall, and feeding and energy storage by the first-stage larvae, are not adequately explained by present descriptions of species of *Philometra*. The objective of this study is to provide a description of the first-stage larvae using electron microscopy. The ultrastructural information provided can then be used to understand better the behaviors of this larval stage.



Figures 1, 2. First-stage larva of *Philometra* sp. from *Aplodinotus grunniens*. 1. Anterolateral view of cephalic end. a, amphidial pore; e, egg tooth; lr, labial ridges; ob, oral bars; p, pseudolabium. Scale bar = 1.1 μm. SEM. 2. Caudal end. c, cuff. Scale bar = 0.7 μm. SEM.

Materials and Methods

Small (120–160-mm) freshwater drum, *Aplodinotus grunniens,* were collected by otter trawl in Lake Erie's western basin off South Bass Island, Ohio. Infected fish were readily apparent because they exhibited popeye. This condition, characterized by protruding, reddened eyes, was seen from late June to early August in freshwater drum from the western basin. Upon dissection, 1 to several red, coiled, larvigerous female *Philometra* sp. were found in the ocular orbits of these fish. These worms rupture on exposure to lake water releasing first-stage larvae. After a short period of time to allow for any changes that might occur on release into lake water, the larvae were collected by centrifugation and fixed in 6% gluteraldehyde in Millonig's phosphate buffer according to a technique modified from Martinez-Palomo and Martinez-Baez (1977). Two percent DMSO was used to facilitate penetrance of the fixative through the cuticle of the larvae. After alcohol dehydration, the larvae were placed in 100% propylene oxide and then embedded in thin films of Spurr's (1969) low-viscosity resin. Specific larvae were excised, oriented, and reembedded onto epoxy blocks for serial sectioning.

Serial cross and longitudinal sectioning was done on an LKB NOVA ultramicrotome using glass and diamond knives. Sections showing silver interference colors (60–90 μm) were picked up on uncoated 200-mesh grids, stained with uranyl acetate (Watson, 1958) and lead citrate (Reynolds, 1963), and examined on a Zeiss 109R transmission electron microscope (TEM) operated at 80 kU.

Glutaraldehyde-fixed larvae were processed for scanning electron microscopy (SEM) using critical-point drying following alcohol dehydration. The larvae were placed on an adhesive-coated stub and coated with gold. They were scanned on a Hitachi S-500 SEM. To be sure that the head and tail structures seen using light microscopy (LM) and SEM were not artifacts of fixation, living larvae were examined under LM using Nomarsky differential interference optics.

Results

Living first-stage larvae of *Philometra* sp. have a concave, oval-spatulate posterior end that resembles a hook from the side. Anterior to this structure, the tail narrows to a small cuff. One large papillalike structure is present on the cephalic end of the larvae. No other structures were discernable with LM.

SEM of *Philometra* sp. reveals the presence of a single papillalike egg tooth located dorsally on the cephalic end of the first-stage larva (Fig. 1). There are labial ridges dorsal and ventral to the mouth and pseudolabia are present laterally (Fig. 1). Lateral amphidial pores are present on the pseudolabia. A pair of barlike structures, arranged laterally, lie within the oral opening (Fig. 1). The cuticle is striated and without ornamentation. An opening, presumably the excretory pore, is present on the ventral surface about 75–100 μm from the posterior end. The caudal area is oval-spatulate and appears to be flexible. Anterior to this structure, the tail narrows to a small cuff (Fig. 2).

Cross sections of the cephalic region, when

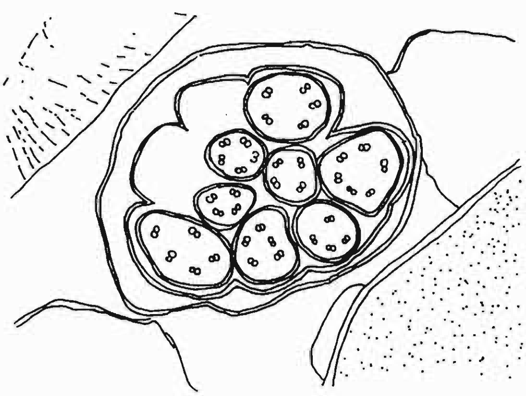


Figure 3. First-stage larva of *Philometra* sp. from *Aplodinotus grunniens.* Lateral ciliary canal drawn from an electron micrograph (TEM).

viewed using TEM, show sensory cilia associated with the amphidial pores, with 8 cilia located in a membrane-lined ciliary canal that exits anteriorly through an opening in the cuticle (Fig. 3). These cilia do not appear to possess basal bodies and are aberrant in that they show variation in microtubule pattern similar to those described for *Dirofilaria immitis* (Kozek, 1968). The egg tooth is also associated with a sensory cilium. This cilium is located primarily within the cuticle and is not contained in a ciliary canal (Fig. 4). The egg tooth appears to be cuticular and does not appear to be associated with secretory cells.

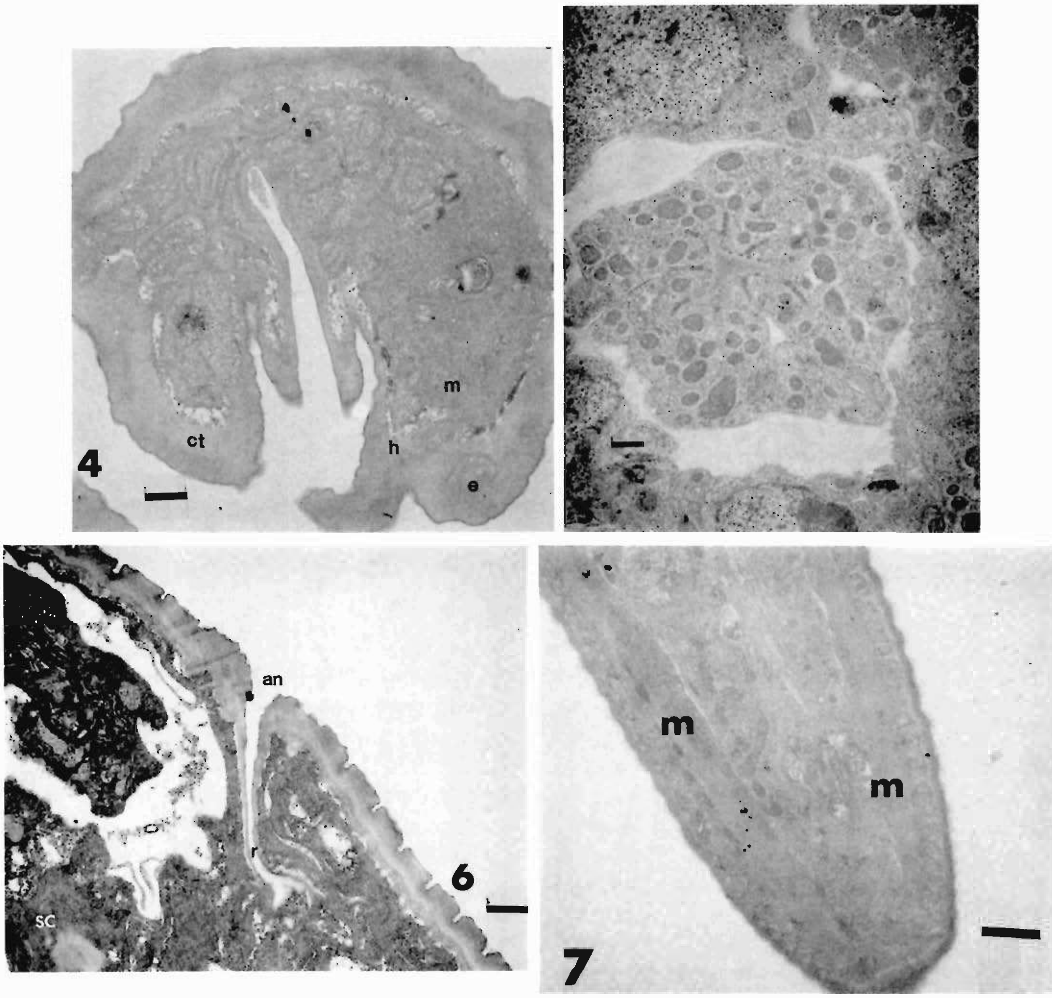
Longitudinal sections through the cephalic region show that the oral bars seen with SEM contain hypodermis and muscle and are lined with cuticle that is continuous with the external cuticle (Fig. 4). The cuticle lining the oral bars also lines the open esophagus. The lumen of the esophagus is filled with granular yolklike material. It continues into an area further posterior in the digestive tract that is not lined with cuticle and probably represents the beginning of the intestine.

Externally, a striated cuticle about 0.17 μm thick is present. Underlying the cuticle is the hypodermis, which is arranged in dorsal, ventral, and lateral cords. The hypodermis of the first-stage larvae appears to be cellular with large nuclei present, although in later stages the hypodermis is syncytial. Each lateral cord contains a honeycomblike structure that may be part of the excretory system, and there are nervous elements, consisting of tightly packed membranes, present. Fixation distortion and other fixation-related problems account for much of the inability to find definite structure in the hypodermis. The circumesophageal nerve ring was not apparent in any of the sections taken, but it is visible in LM of living and fixed larvae. Four rows of muscle cells, running anterior to posterior, are present immediately beneath the hypodermis, with 2 cells present ventrally and 2 dorsally. Each has a cytoplasmic region containing a large nucleus and a contractile region containing longitudinally arranged myofilaments typical of active muscle. The esophageal lumen of the first-stage larvae is surrounded by 3 or 4 cells arranged in a single layer (Fig. 5). The open, yolk-filled lumen of the esophagus ends in a triradiate structure that is without an apparent lumen (Fig. 5) and again opens up into a yolk-filled area that is not lined with cuticle. The digestive tract, posterior to this dilated open region, is undeveloped and closed. Three or 4 cells surround a long, narrow intestinal thread having no apparent lumen situated at the midsection of the digestive tract. Posterior to this is an area predominated by storage cells filled with granular material. Beyond the storage cells are columns of 2 or 3 cells, each containing a large nucleus and many mitochondria. Among these cells is a cuticle-lined rectum that does not appear to be connected to the digestive tract (Fig. 6) but that leads into a cuticle-lined anus. Rows of muscle are present at the caudal end (Fig. 7). No secretory tissue was found in the caudal end, nor were any pores, bristles, or other structures of attachment seen. The phasmids were not apparent in any of the sections, nor were phasmidial pores observed using SEM.

Discussion

Philometrids are ovoviviparous. The zygote develops to a vermiform stage enclosed in an egg membrane within the uterus of the female. These embryos increase in size as they develop and appear to store lipids and glycogen (Crites, 1980). First-stage larvae eventually rupture through the egg membranes and lie free in the uterus until released into the water by the bursting of the female worms (Crites, 1980).

Morphological features observed in this study for the first-stage larvae of *Philometra* sp. are an elongate oral opening containing oral bars and surrounded by 2 lateral pseudolabia bearing amphids and 2 lateral ridges, 1 bearing a dorsal egg tooth; an open lumen to the esophagus leading into an open portion of the digestive tract that is not lined with cuticle; an intestinal thread sur-



Figures 4–7. **First-stage larva of *Philometra* sp. from *Aplodinotus grunniens*. 4. Oblique section through anterior end. ct, cuticle; h, hypodermis; m, muscle. Scale bar = 0.4 μm. TEM. 5. Cross section through esophageal-intestinal junction. Scale bar = 1.4 μm. TEM. 6. Oblique section through posterior region of digestive tract. an, anus; r, rectum; sc, storage cells. Scale bar = 0.7 μm. TEM. 7. Longitudinal section through posterior end. Scale bar = 1.4 μm. TEM.**

rounded by cells leading into an area of storage cells; a column of cells surrounding a cuticle-lined rectum that leads into an anus; and a long tapering tail ending in an oval-spatulate structure (Fig. 8). It is apparent from SEM of the first-stage larvae that major differences, most likely related to life-style and habitat, occur between adult and larval forms. In contrast to the adult philometrids, which have 2 circles of papillae surrounding the oral opening, only 1 unpaired papilla is present on the cephalic end of the first-stage larvae. This egg tooth is common on many larvae that use copepod intermediate hosts. First-stage larvae of nematodes of the superfamily Dracunculoidea were described as having a cephalic dorsal denticle by Chitwood and Chitwood (1950). Li (1935) described the larvae of *Procamallanus fulvidraconus* as being provided with a dorsal spine for penetrating the copepod intestinal wall. Hedrick (1935), in his study on the life history of *Spiroxys contortus* (Nematoda: Spiruridae), stated that the larva penetrates the body cavity of cyclops (Copepoda) with the aid of a cuticular tooth. Furthermore, he assumes the process to be mechanical, because no glands or ducts can be seen leading to the base of the tooth. When

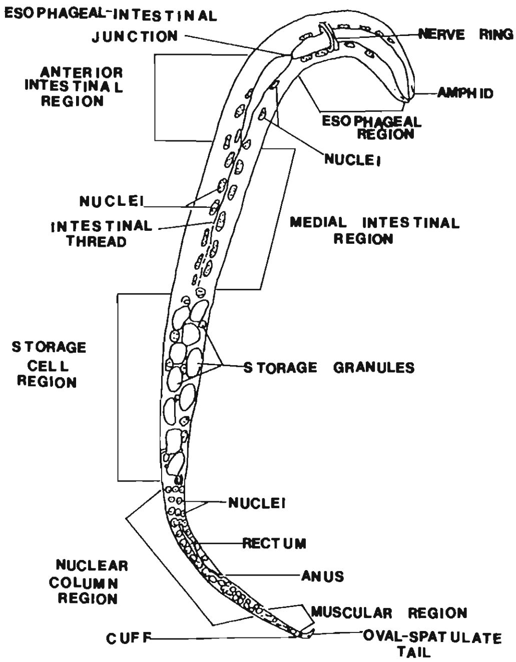


Figure 8. Diagrammatic representation of the first-stage larva of *Philometra* sp. from *Aplodinotus grunniens*.

the larva molts, this egg tooth is shed with the cuticle (Hedrick, 1935). The egg tooth of *Philometra* sp. first-stage larvae is cuticular and does not appear to be associated with any secretory cells.

Amphids, present on the adult of *Philometra* sp., are also found as early in development as the first-stage larvae. The amphids contain cilia enclosed in ciliary canals, similar to those described by Kozek (1968) in *D. immitis* (Nematoda: Filarioidea). The egg tooth also appears to be associated with a subsurface sensory cilium, indicating that it may have mechanoreceptor function beyond its supposed function in penetrating the copepod gut wall. This cilium appears to be located primarily in the cuticle and is not enclosed within a canal. Like the sensory cilia of the amphids, the cilium of the egg tooth is varied in microtubule pattern and does not appear to have a basal body. The egg tooth cilium may be used in orientation of the larva with respect to the copepod gut wall.

Mention of cuticular projections in the family Spiruridae has been made by Chitwood and Wehr (1934), but the philometrids were distinguished from the spirurids by lack of such structures. However, the microfilariae of members of the superfamily Filarioidea also have cuticular structures associated with the oral opening. These structures are described by Laurence and Simpson (1968) as a cephalic space containing an eversible hook and spines attached to an oral ring supplied with muscle for movement. These structures are lost at first molt. It is possible that larval cephalic structures of *Philometra* sp. may provide further evidence of evolutionary continuity within the order Spirurida and may provide a link with the Filarioidea. However, other larval stages need to be studied.

The oral opening of the first-stage larvae of *Philometra* sp. is elongate and contains oral bars. The oral bars open externally into the oral opening. These structures are not found in the adult, although adult structure is disputed. The purpose of the oral bars is unknown. The mouth of the first-stage larva of *Philometra* sp. opens into a cuticle-lined esophagus. The cuticular lining is continuous with the cuticle of the oral bars, suggesting that these are the ends of a buccal capsule. The esophageal lumen is filled with yolk, apparently a remnant of the yolk supplied to the embryo before the egg membranes are laid down. The cuticular lining ends halfway down the digestive tract, indicating that at least part of the intestine is also developed. Taylor (1960) described the intestine of the filarid *Dirofilaria immitis* as starting as a solid mass of cells that later form a cavity through the center. Development of *Philometra* sp. appears to be similar. Posterior to this open area is a column of cells surrounding an intestinal thread similar to the pharyngeal thread, or long narrow tube that the gut develops around, described by McLaren (1972) for various filarid species. However, while the pharyngeal thread is lined with cuticle anteriorly where the esophagus will form, the intestinal thread is not lined with cuticle anywhere along its length, indicating that the esophagus has already differentiated but the intestine, which is not lined with cuticle, has not—a somewhat more advanced stage of development than has been described for the filarids.

The posterior end of the intestinal area is filled with storage cells. Histochemical staining of this area shows these cells to be periodic acid–Schiff positive, indicating the presence of glycogen, and Sudan B-black positive, indicating the presence of lipids (Crites, 1980). Von Brand (1966) indicated that granules in the digestive tract provide

an endogenous energy source for the larvae. Given the large amount of stored energy within the larvae themselves, and the lack of a completely differentiated digestive system, it is likely that the larvae do not feed at this stage. An anus and a rectum are present, although by the time the third larval stage is reached the rectum has already atrophied (Crites, 1980). In larvigerous females, the anus is absent and the rectum has degenerated to a strand that connects the end of the intestine to the body wall (Crites, 1980), indicating that secretion of solid wastes probably does not take place at this stage either, although the adult females do feed on red blood cells (Crites, 1980). Whether the anus and rectum are ever functional in the philometrids, or they are only present vestigially as part of the developmental sequence, is unknown.

Philometra sp., as a member of the class Secernentia, should have phasmidial pores opening near the anus. Phasmids were not seen on any of the first-stage larvae examined and are possibly not present until a later developmental stage. Phasmids are present on adult philometrids and have been described for the larvae of *Philometra obturans* (Moravec, 1980).

Crites (1980) describes clumping behavior of the first-stage larvae and indicates that although larvae are capable of attachment by their tails, no glands could be ascertained. Neither SEM nor TEM produced evidence of cement glands, bristles, spines, or secretory cells in the caudal end of the first-stage larvae of *Philometra* sp. However, rows of muscle were found, indicating that the oval-spatulate area of the tail may function as a sucker or grasping organ. No mention has been made of such organs in any other larval stages studied, nor are suckers and muscular grasping organs a common adaptation of nematodes.

From a behavioral standpoint, clumping by larvae may increase the probability of their being encountered by copepods. This would be important to an organism that is relying on a finite amount of stored energy, and it is indicated by Stromberg and Crites (1974) that copepods are more strongly attracted to larvae that are moving. Larvae continue to be active for up to 27 days depending on temperature, but infectivity decreases rapidly in this time (Crites, 1980). Activity of the larva and ability to penetrate the copepod gut wall are significantly correlated, indicating that larval activity is important to gut wall penetration (Stromberg and Crites, 1974). This is substantiated by the lack of visible secretory structures associated with the egg tooth of the first-stage larvae.

This study provides a more complete description of the first-stage larva of *Philometra* sp. and provides a basis for the understanding of some of the larval behavior. There is also the possibility that this description of the larvae will help in assigning evolutionary relationships among the spirurids and provide a better understanding of the relationship between the philometrids and filarids. It does point to the importance of knowing larval structure and poses questions about the developmental processes occurring in the other larval stages and in the adults of *Philometra* sp. and related genera.

Acknowledgments

We thank The Ohio State University and F. T. Stone Laboratory for use of their facilities.

Literature Cited

Chitwood, B. G., and M. B. Chitwood. 1950. Introduction to Nematology. University Park Press, Baltimore, Maryland. 334 pp.

———**, and E. E. Wehr.** 1934. The value of cephalic structures as characters in nematode classification. Zeitschrift für Parasitenkunde 7:273–335.

Crites, J. L. 1980. Project F-48-R-2, #1. Impact of *Philometra* sp. on Lake Erie fishes. C.L.E.A.R. Technical Report #192. 344 pp.

Hedrick, L. R. 1935. Life history and morphology of *Spiroxys contortus.* Transactions of the American Microscopical Society 54:307–335.

Kozek, W. J. 1968. Unusual cilia in the microfilaria of *Dirofilaria immitis.* Journal of Parasitology 54: 838–844.

Krecker, F. H. 1916. *Filaria cingula* parasitic in the skin of *Cryptobranchus allegheniensis.* Journal of Parasitology 2:74–79.

Laurence, B. R., and M. G. Simpson. 1968. Cephalic and pharyngeal structures in microfilaria revealed by staining. Journal of Helminthology 34:27–38.

Li, H. C. 1935. The taxonomy and early development of *Procamallanus fulvidraconus* n. sp. Journal of Parasitology 21:103–113.

Martinez-Palomo, A., and M. Martinez-Baez. 1977. Ultrastructure of the microfilaria of *Onchocerca volvulus* from Mexico. Journal of Parasitology 63: 1007–1018.

McLaren, D. J. 1972. Ultrastructural studies on microfilaria (Nematoda: Filarioidea). Parasitology 65: 317–332.

Moravec, F. 1980. Development of the nematode *Philometra ovata* (Zeder, 1803) in the copepod intermediate host. Folia Parasitologica (Praha) 27: 29–37.

Reynolds, E. S. 1963. The use of lead citrate at high

pH as an electron opaque stain in electron microscopy. Journal of Cell Biology 17:208–212.

Spurr, A. 1969. A low-viscosity epoxy resin embedding medium for electron microscopy. Journal of Ultrastructural Research 26:31–43.

Stromberg, P. C., and J. L. Crites. 1974. The life cycle and development of *Camallanus oxycephalis* Ward and Magath, 1916 (Nematoda: Camallanidae). Journal of Parasitology 60:117–124.

Taylor, A. E. R. 1960. The development of *Dirofilaria immitis* in the mosquito *Aedes aegypti*. Journal of Helminthology 34:27–38.

Thomas, L. J. 1929. *Philometra nodulosa* nov. spec. Journal of Parasitology 15:193–199.

Uhazy, L. S. 1976. Biology of *Philometroides huronensis* n. sp. (Nematoda: Dracunculoidea) of common white sucker (*Catostomus commersoni*). Ph.D., University of Guelph, Ontario.

von Brand, T. 1966. Biochemistry of Parasites. Academic Press, New York. 429 pp.

Watson, M. L. 1958. Staining of tissue sections for electron microscopy with heavy metals. Journal of Biophysical and Biochemical Cytology 4:475–478.

Vertical Transmission of Parasites

Saturday, 1 May 1993

University of Pennsylvania, New Bolton Center, Kennett Square, PA
Annual Joint Meeting with the New Jersey Society for Parasitology

Organizer: Dr. Herbert W. Haines, Animal Drug Evaluation, Basic Animal Science Research, Merck Sharp & Dohme Research Laboratories, R80T-136, PO Box 2000, Rahway, NJ 07065. Tel.: (908) 594-5623; Fax: (908) 594-5700

Speakers:

Dr. Wesley L. Shoop Merck Institute for Therapeutic Research Rahway, NJ 07065-0900	Trematodes
Dr. Ronald Fayer Animal Parasitology Unit U.S. Department of Agriculture Beltsville, MD 20705	Protozoans
Dr. Eugene T. Lyons Dept. of Veterinary Science Gluck Equine Research Center University of Kentucky Lexington, KY 40546-0099	Nematodes
Dr. D. Bruce Conn Department of Biology St. Lawrence University Canton, NY 13617	Cestodes